and diastereomeric analogs of these deoxynucleosides.[15] This synthetic route may also provide access to isotopically labeled nucleosides[16] for structural and pharmacological studies of nucleoside drugs as well as nucleotide polymers DNA and RNA.

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Electron Transfer through DNA: Site-Specific Modification of Duplex DNA with Ruthenium Donors and Acceptors**

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Long-range intramolecular electron transfer (ET) in protein–protein complexes and modified proteins takes place over appreciable distances at biologically significant rates.[1, 2] Experiments have confirmed its dependence on free energy and distance, as well as the importance of the reorganization of the ligand sphere and solvent environment.[3] Two factors that influence the rate of electron transfer that are far less understood in both inorganic and organic systems are the structure of the intervening medium and the orientation of the donor–acceptor unit.

A barrier to the further understanding of the factors that influence the mechanisms of long-range electron transfer is the development of an architecture capable of critically testing these properties. The unique ability of complementary single strands of DNA to hybridize into a rigid duplex of defined structure makes an oligonucleotide an attractive choice for use as a molecular template. This template can be modified with spectroscopically detectable and photochemically active redox centers at predetermined locations. Moreover, recent theoretical work suggests that the electronic coupling between donor and acceptor for modified DNA derivatives could display a remarkable dependence on nucleic acid sequence.[4]

In order to test these assumptions, we have devised a novel approach to prepare ruthenium-modified duplex DNA derivatives where the donor and acceptor are separated by any number of base pairs. The goal of this approach is to prepare a series of ruthenium-modified DNA derivatives in which 1) the ruthenium complexes are covalently and rigidly attached to a single site on the duplex, 2) the redox potential of each ruthenium complex can be varied independently, 3) the DNA duplex is unperturbed by the presence of the ruthenium complexes, and 4) the redox states of the donors and acceptors are spectroscopically distinguishable. This new synthetic procedure covalently attaches redox centers of varying potentials to a series of oligonucleotides each modified with a primary amino group at the 2′ position of the 5′-terminal ribose (Fig. 1). Intramolecular ET rates obtained from these rigidly attached redox centers should provide insight into the electronic coupling between donor and acceptor linked to DNA.

Our strategy for the preparation of these transition metal DNA derivatives involves a departure from previous work in which oligonucleotides have been modified with organic molecules and metal complexes at the terminal phosphates,[5–6] various heterocyclic bases,[7–11] and the ribose ring.[12–13] This new approach employs the synthesis of two sets of complementary strands (8–14 base pairs in length) of oligonucleotides modified with a terminal aminoribose[14] and subsequent covalent modification with redox-active ruthenium complexes. Unlike fluorescent DNA tags such as fluorescein isothiocyanate

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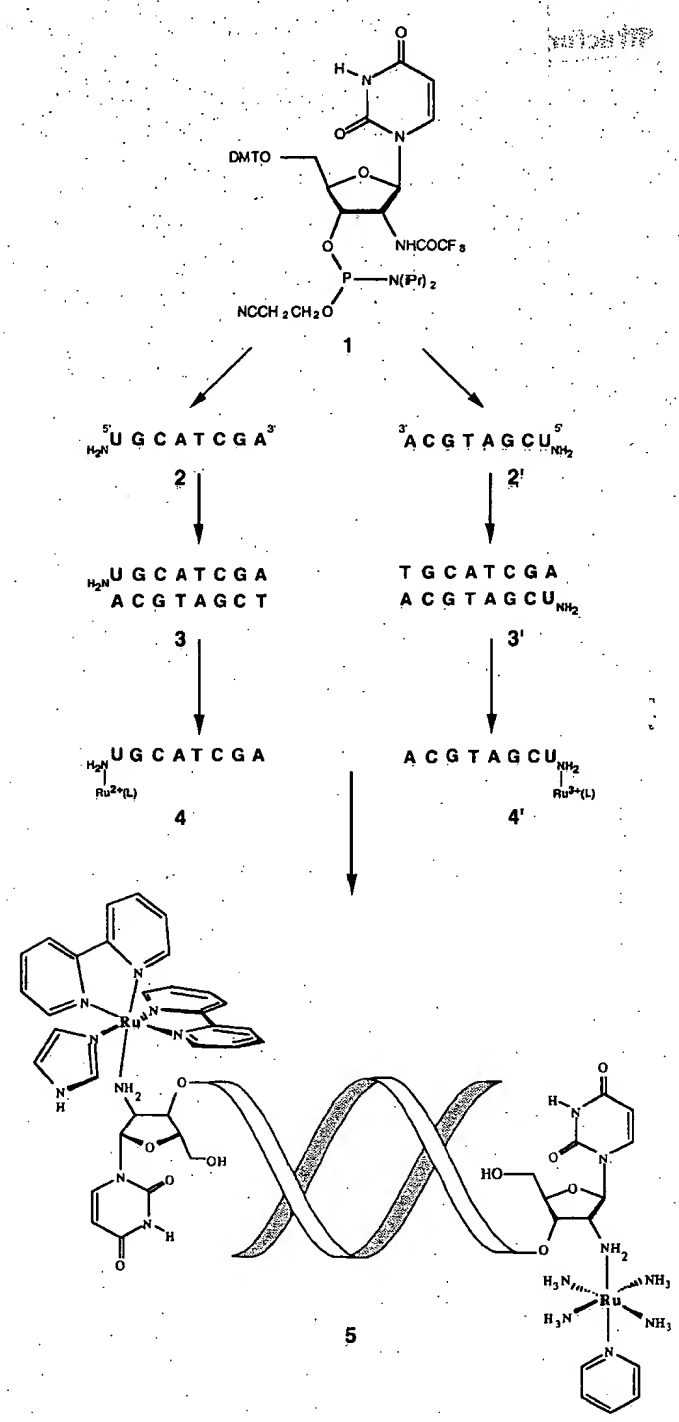

Fig. 1. Scheme for the preparation of duplex DNA labeled with donors and acceptors. The DMT-2'-*N*-trifluoroacetyl-protected phosphoroamidite (**1**) is incorporated into an oligodeoxyribonucleotide (**2**, **2'**) by automated solid-phase synthesis techniques and "protected" with a complementary strand (formation of 3/3'). After reaction with a ruthenium complex, the complement is removed, and the modified oligonucleotide purified. The procedure is repeated and the strands annealed to yield **5**. Abbreviations U_{NH_2} = 2'-amino-2'-deoxyuridine; L = 2,2'-bipyridine, imidazole, pyridine, NH_3; DMT = dimethoxytrityl.

(FITC), the ruthenium complexes used in this work will react with the heterocyclic nitrogen atoms of the bases. Therefore, an unmodified complementary sequence is used as a large hydrogen-bonded blocking group to protect the sites on the bases that are susceptible to attack by the metal.[15] Under these conditions, the exposed primary 2'-amine at the 5'-terminal ribose position of the duplex DNA is readily modified with a variety of ruthenium complexes. In contrast, random ruthenium modification of the heterocyclic nitrogen centers is eliminated in some cases and substantially reduced in others.

A DMT-2'-*N*-trifluoroacetyl-protected phosphoroamidite (**1**) of 2'-amino-2'-deoxyuridine (U_{NH_2}) was prepared by variation of published procedures,[12] and oligodeoxyribonucleotides (**2**/**2'**) were assembled by standard solid-phase automated DNA synthesis techniques.[16] The 2'-amino-oligodeoxyribonucleotide **2** was annealed to the complementary sequence to form **3**. This U_{NH_2}-containing duplex was treated with $[Ru(bpy)_2CO_3]$, followed by imidazole in an inert atmosphere. The recovered duplex DNA was denatured in 7 M urea, and the ruthenium-modified oligonucleotide[17] was purified by using C-18 reversed-phase HPLC techniques (Fig. 2). This procedure was repeated with the complementary strand **2'** employing $[Ru(NH_3)_4(py)]^{2+}$ as the acceptor. Finally, the two ruthenium-modified oligonucleotides **4** and **4'** were annealed to give the desired duplex DNA **5** with a covalently bound donor and acceptor.

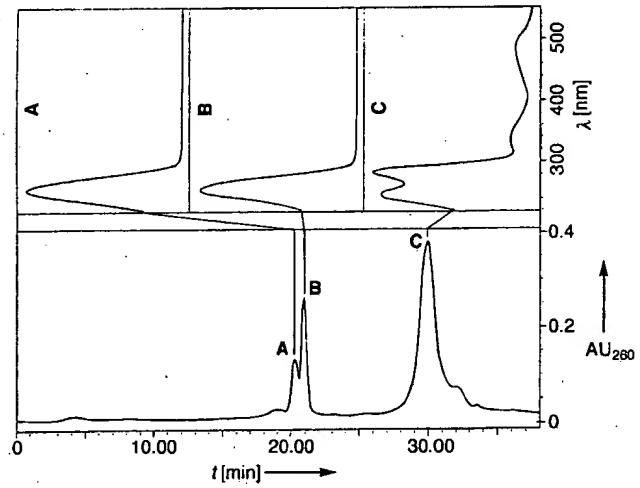

Fig. 2. HPLC analysis and UV/Vis spectra of duplex DNA following modification with ruthenium. Oligonucleotides were denatured in 7 M urea at 60 °C, injected onto a C-18 reversed-phase column, and eluted with a solvent mixture with a gradient of 2 to 40% CH_3CN in 7 M urea and 0.1 M triethylammonium acetate, pH 6.5. Peak A (25.4% CH_3CN) is unchanged amino-modified oligonucleotide (U_{NH_2}GCATCGA); peak B (27.7% CH_3CN) is the oligonucleotide complement of A (ACGTAGCT); peak C (38.8% CH_3CN) corresponds to Ru-modified A ($U_{NH_2Ru(bpy)_2}$(im)GCATCGA), where the absorption spectrum is consistent with the presence of a single $Ru(bpy)_2(im)(U_{NH_2})$ complex covalently bound to the oligonucleotide. Identities of peaks A, B, and C have been confirmed by coinjections of authentic samples and by enzymatic digestion (alkaline phosphatase, phosphodiesterase) followed by HPLC analysis of the nucleoside composition.

The new metal-modified oligonucleotides were characterized by fluorescent labeling, enzymatic digestion, and duplex-melting temperature studies. The aminoribose oligomers and their complements were treated with FITC under conditions that favor labeling of primary amines. As expected, only the 2'-amino-2'-deoxyribose site was labeled, which verified the presence of a primary amine on the DNA. Thermal denaturing and annealing experiments display similar melting temperatures for both ruthenium and aminoribose oligomers.[18] In addition, the amino-modified duplex DNA has been characterized by 2D NMR.[19] These data confirm that the donors and acceptors are covalently attached to the 2'-amino-2'-deoxyribose position and indicate that the DNA structure is unperturbed by the presence of the ruthenium complexes.

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Kinetic measurements were performed by direct photoinduced and flash-quench techniques.[20] The rate of intramolecular electron transfer in the eight base-pair long duplex, modified with the acceptor $[Ru(bpy)_2(im)]^{3+}$ and the donor $[Ru(NH_3)_4(py)]^{2+}$, was determined to be $1.6(4) \times 10^6\ s^{-1}$ (metal–metal distance = 21 Å).[21] Since the driving force for this reaction ($-\Delta G^0 \approx 0.7$ eV) is well below the estimated reorganization energy ($\lambda \approx 0.9$ eV),[3, 20, 22] the activationless rate constant for electron transfer between ruthenium centers is expected to be roughly $2.5 \times 10^6\ s^{-1}$. Interestingly, this estimated k_{max} value is comparable to the k_{max} of one of the most efficient protein systems studied, His39-modified cytochrome c (Fe-to-Ru distance = 20.3 Å).[23]

Additional rates are required to determine the distance-dependent nature of this process, as well as to delineate the roles of base sequence and π-stacking in modulating the rates of long-range electron-transfer reactions through DNA.

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[18] Temperatures at which 50% of the DNA strands are dissociated (T_m) were determined by thermal denaturation and annealing in 0.9 M NaCl and 0.1 M Na phosphate buffer (pH 7) by means of a Hewlett Packard 8452A diode-array spectrophotometer with HP 89090A Peltier temperature-control accessory. Unmodified octamers as controls revealed a single helix-to-coil transition point and a T_m of 45 °C. The aminoribose oligomers showed a lower T_m of 40 °C. The ruthenium-modified oligomers, when hybridized to their unmodified complements, showed broad helix-to-coil transitions between 36 °C and 42 °C. All kinetic measurements were performed in the presence of 1 M Na^+ to insure that the modified oligonucleotides were annealed at ambient temperature (22 °C).
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Structure and Bonding of Transition Metal Dihydrogen Complexes $[M(CO)_5(H_2)]$ (M = Cr, Mo, W)**

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Dedicated to Professor Paul von Ragué Schleyer on the occasion of his 65th birthday

Since the first synthesis of a stable dihydrogen complex, $[W(CO)_3(PR_3)_2(\eta^2\text{-}H_2)]$ (R = c-C_6H_{11} (Cy), *i*Pr) was reported by Kubas et al.[1] in 1984,[1a] many theoretical and experimental studies on the structures and properties of transition metal dihydrogen complexes have been published.[2] Although metal dihydrogen complexes play an important role as intermediates in catalytic hydrogenation reactions, relatively little is known about their geometries and bond strengths. The exact experimental determination of the equilibrium geometry of the L_nM-H_2 moiety is difficult, because the very low barrier for rotation about the $M-H_2$ axis even at very low temperatures yields a high degree of librational motion, which artificially shortens the H–H bond.[2, 3] Neutron diffraction studies of several complexes showed surprisingly similar H–H distances of 0.82 Å,[2–4] but recent experimental investigations of dihydrogen complexes reported H–H distances of >1 Å.[5]

Even more difficult than the geometry is the experimental determination of the $M-H_2$ bond energy. Ishikawa et al.[6a] estimated from studies of the lifetimes of several $[W(CO)_5L]$ complexes a W–L binding energy of >16 kcal mol^{-1} for $L = H_2$. The metal–ligand binding enthalpy of H_2 in $[W(CO)_3(PCy_3)_2(H_2)]$ was determined by Gonzalez et al.[6b] by using solution calorimetry as $\Delta H = 9.9$ kcal mol^{-1}. A quantum mechanical study of $[Cr(CO)_5(H_2)]$ by Pacchioni[7] gave a dissociation energy of 9.6 kcal mol^{-1} for H_2 dissociation. However, this value was derived from calculations only at the Hartree–Fock level of theory with partially optimized geometries.

The parent carbonyl complexes $[M(CO)_5(H_2)]$ are important for the study of $M-H_2$ bonding, because the unsubstituted molecules make it possible to analyze the metal–dihydrogen interactions in detail. The chromium analogue $[Cr(CO)_5(H_2)]$ is of particular interest, as it is a postulated intermediate in the water gas shift reaction.[8] The complexes $[M(CO)_5(H_2)]$ (M = Cr, Mo, W) have been characterized in noble gas matrices by IR spectroscopy,[9] but the equilibrium geometries and bond energies are not known. We have recently shown that the metal–ligand bond lengths calculated at the MP2 level of theory and the theoretically predicted first dissociation energies at the CCSD(T) level of theory using effective core potentials for the metals in complexes $[M(CO)_5L]$ (M = Mo, W; L = CO, CS) are in excellent agreement with experimental values, while the calculated Cr–L interatomic distances of $[Cr(CO)_5L]$ are slightly too short and the first dissociation energies too high.[10] Similar results have been obtained for $[M(CO)_5]$ (M = Fe, Ru, Os) and $[M(CO)_4]$ (M = Ni, Pd, Pt).[11] In this paper we report the theoretically predicted equilibrium geometries, vibrational frequencies, and metal–dihydrogen bond energies of $[M(CO)_5(H_2)]$ (M = Cr, Mo, W) obtained at the same theoreti-

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